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# Reverse Transcriptase PCR Detection of Astrovirus, Hepatitis A Virus, and Poliovirus in Experimentally Contaminated Mussels: Comparison of Several Extraction and Concentration Methods

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**Four methods of extraction and three methods of concentration of three enteric viruses from mussels were comparatively evaluated by reverse transcriptase PCR (RT-PCR). Shellfish were experimentally contaminated by immersion in seawater seeded with astrovirus, hepatitis A virus, or poliovirus. Sixty-gram samples of mussel tissues were processed by using borate buffer, glycine solution, saline beef, and saline beef-Freon extraction methods. The viruses were concentrated by precipitation with polyethylene glycol 6000 (PEG 6000) or PEG 8000 or by organic flocculation. RT-PCR was performed with RNA extracts from crude shellfish extracts and concentrates with and without Sephadex LH20 filtration. The glycine solution and borate buffer extraction methods resulted in significantly more RT-PCR-positive samples than the saline beef extraction method. We assessed the efficiency of 20 combinations of extraction and concentration methods. The borate buffer-organic flocculation, borate buffer-PEG 6000, and glycine solution-PEG 6000 combinations gave RT-PCR-positive results for all 27 samples analyzed for the three viruses. Detoxification of the samples by Sephadex LH20 filtration significantly decreased the efficiency of RT-PCR virus detection.**

Enteric virus contamination of shellfish harvested for human consumption is a public health concern. Outbreaks of gastroenteritis have occurred among consumers of raw or undercooked shellfish harvested from fecally polluted waters (9, 10, 13, 18, 21–23). Detection of enteric viruses in shellfish involves viral extraction from the shellfish tissues and viral concentration. Detection by cell culturing is slow and expensive, and most of the epidemiologically important enteric viruses are either difficult to cultivate or noncultivatable. PCR offers the best alternative for developing sensitive and specific tests for detection of enteric viruses in shellfish (3, 7, 9, 12, 17), but in environmental samples interference by PCR inhibitors may occur (3). Concentration and purification of virions from shellfish rely on physicochemical procedures (1, 6, 15, 17, 20, 26). Some methods have been tested to evaluate their efficiency for removing amplification-inhibiting agents from shellfish (3, 7, 14, 17). However, a single, simple method that is efficient for multiple viruses is still needed. The aim of this study was to compare four viral extraction methods, the borate buffer (6), glycine solution (20, 26), saline beef (1), and saline beef-Freon (1) extraction methods, and three virus concentration methods, the polyethylene glycol 6000 (PEG 6000) (20) and PEG 8000 (1) precipitation and organic flocculation (OF) (15) methods. In addition to astrovirus and hepatitis A virus (HAV), two clinically important enteropathogens, we studied poliovirus because it has been used to evaluate most of the methods included in this study. The viruses were detected by reverse transcriptase PCR (RT-PCR) in mussels contaminated under simulated natural conditions. A method for detoxification of mussel extracts (Sephadex LH20 gel filtration) was also tested to determine its ability to remove PCR inhibitors.

Astrovirus reference strain HAstV1 was kindly provided by Stephan Monroe, Centers for Disease Control and Prevention (Atlanta, Ga.). HAV strain CF 53 was supplied by J. M. Crance (Centre de Recherche du Service de Santé des Armées, La Tronche, France). Poliovirus type 1 strain LSc 2 ab was propagated in Buffalo green monkey kidney cells. The mussels (*Mytilus edulis*) used in the experiments came from a sea farm on the French Atlantic coast. Before use, they were stored in a 280-liter seawater basin at 16°C for at least 4 days. They were contaminated by immersion in seawater (Reef Crystal; Aquarium System, Sarrebourg, France) for 1 h in 10-liter tanks experimentally seeded with astrovirus (final concentration, $3 \times 10^4$ PFU/ml), HAV (final concentration, $9 \times 10^3$ 50% tissue culture infective doses/ml), or poliovirus (final concentration, $3.2 \times 10^3$ most probable number of cytopathogenic units [MPNCU]/ml). The mussels were then rinsed in deionized water, shucked, and drained of excess fluid, and their tissues were stored in 60-g aliquots at −20°C before further processing (11).

Four extraction methods (the borate buffer, glycine solution, saline beef, and saline beef-Freon extraction methods) were performed as a first step. Each mussel extract was then processed with two or three concentration methods (the OF, PEG 6000, and PEG 8000 methods) depending on the extraction method. One-half of each final concentrate was directly analyzed by RT-PCR, and the other half was detoxified by Seph-

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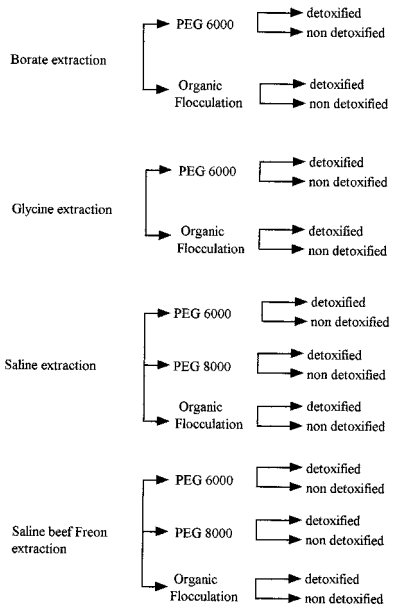


FIG. 1. Procedures used for extraction and concentration of viruses from mussel samples. Detoxified samples were filtered through a Sephadex LH20 gel.

adex LH20 gel filtration before RT-PCR analysis. This protocol is outlined in Fig. 1.

**Virus extraction.** The borate extraction method was performed as described by Boher and Schwartzbrod (6). Briefly, the mussel tissues were ground in a Waring blender at 10,000 rpm for 3 min and mixed with 100 ml of 1 M borate–3% beef extract buffer (pH 9). The suspension was homogenized with an Ultraturrax homogenizer at 9,500 rpm for 1 min and stirred magnetically for 15 min. The suspension was then sonicated for 1 min at 100 W and finally was clarified by centrifugation at 10,000 × *g* for 90 min at 4°C.

For glycine extraction (20, 26), mussel tissues were homogenized with an Ultraturrax homogenizer at 9,500 rpm for 3 min in 50 ml of 0.05 M glycine–0.15 M NaCl buffer (pH 9). The suspension was stirred magnetically for 15 min and then centrifuged at 5,000 × *g* at 4°C for 10 min. The saline beef extraction method was performed by using a described previously procedure (1). The mussels were crushed in 50 ml of a 0.3 M NaCl solution with an Ultraturrax homogenizer at 9,500 rpm for 1 min. Then 350 ml of an eluting solution containing 0.3 M NaCl and 7% beef extract (pH 7.5) was added. The mixture was homogenized again with the Ultraturrax homogenizer at 9,500 rpm for 1 min and centrifuged at 5,000 × *g* for 20 min at 4°C.

For saline beef-Freon extraction, mussels were processed as described above for the other extraction methods, and then 100 ml was reextracted by mixing it with an Ultraturrax homogenizer at 9,500 rpm for 1 min with an equal volume of Freon (1,1,2-trichlorotrifluorethane; Sigma Chemical Co., St. Louis, Mo.) and centrifuging it at 5,000 × *g* for 20 min at 4°C. The pH of the supernatant was adjusted to 7.2. In all four extraction procedures the supernatants were the viral extracts.

**Virus concentration from mussel extracts.** Viral concentration by OF of the mussel extract was accomplished by lowering the pH to 3.5 with stirring for 30 min. The pellet obtained after centrifugation at 3,000 × *g* for 10 min was resuspended in 12 ml of 0.15 M $Na_2HPO_4$ (pH 9). The suspension was clarified by centrifugation at 1,500 × *g* for 20 min at 4°C, and the pH was adjusted to 7.2 (15).

For PEG 6000 precipitation, we used a simplified version of a previously described method (20). The pH of the extract was adjusted to 7.3, and the extract was supplemented with 10% (final concentration) PEG 6000 and incubated overnight at 4°C. The precipitated viruses were recovered by centrifugation at 10,000 × *g* for 90 min at 4°C. The pellet was resuspended in 12 ml of $Na_2HPO_4$ (pH 9) with vigorous magnetic stirring. The suspension was clarified by centrifugation at 1,500 × *g* for 20 min at 4°C, and the pH was adjusted to 7.2. For PEG 8000 precipitation, the mussel extract was supplemented with 12% (final concentration) PEG 8000 and incubated overnight at 4°C. The precipitated viruses were recovered by centrifugation at 6,200 × *g* for 20 min at 4°C and were resuspended in 12 ml of $Na_2HPO_4$ (pH 9). The suspension was clarified by centrifugation at 1,500 × *g* for 20 min at 4°C, and the pH was adjusted to 7.2 (1). One-half of each concentrate (6 ml) was detoxified by a previously described method based on filtration through a Sephadex LH20 gel (Pharmacia Biotech) (5).

**PCR procedures.** Total RNA extraction was performed by using 200-μl portions of mussel concentrates or extracts and an RNA-PLUS purification kit (Bioprobe Systems, Montreuil, France) according to the manufacturer's instructions. The RNA pellet was resuspended in 25 μl of diethyl pyrocarbonate-treated water for astrovirus and in 150 μl of diethyl pyrocarbonate-treated water for HAV and poliovirus. Total RNA extraction was performed by using undiluted samples and samples diluted 10-fold with sterile water.

**PCR primers.** Primers MON 340 (5′ CGTCATTATTTGT TGTCATACT 3′) and MON 348 (5′ ACATGTGCTGCTGT TACTATG 3′), which were used for the astrovirus RT-PCR, are located in open reading frame 1A and yield a 289-bp amplicon (4). The following primers used for the poliovirus RT seminested PCR were from the 5′ noncoding region: primer 2 (5′ CAAGCACTTCTGTTTCCCCGG 3′), primer 3 (5′ ATT GTCACCATAAGCCA 3′), and primer F2 (5′ CTTGCGCGT TACGAC 3′) (19). The resulting fragment was 366 bp long. The HAV RT-PCR primers were derived from an HAV conserved DNA sequence coding for capsid proteins VP1 and VP3. The 39-nucleotide primers, primer D (5′ GTTTTGCTCCTCT TTATCATGCTATGGATGTTACTACAC 3′) and primer E (5′ GGAAATGTCTCAGGTACTTTCTTTGCTAAAACTG GATCC 3′), yield a 248-bp amplicon (2).

**RT-PCR analysis of viral RNA in mussel extracts and concentrates.** For astrovirus, 5 μl of RNA solution was added to 20 μl of an RT mixture containing 10 U of avian myeloblastosis virus RT (Promega, Madison, Wis.), 5 μl of 5× enzyme buffer (Promega), 2 μl (each) of four 10 mM deoxynucleoside triphosphate stock solutions (Boehringer Mannheim, Indianapolis, Ind.), and 25 pmol of RT primer MON 348, and the mixture was incubated for 1 h at 42°C. The PCR was performed by using 5 μl of cDNA along with 0.5 U of *Taq* DNA polymerase (Appligene, Illkirch, France) and 25 pmol of each primer in a final volume of 50 μl. Denaturation was performed for 3 min at 94°C, and this was followed by 30 cycles of amplification consisting of denaturation for 30 s at 94°C, annealing for 20 s at 50°C, and extension for 30 s at 72°C. A final extension step was performed for 5 min at 72°C.

The poliovirus RT reaction was performed with oligo$(dT)_{15}$ (Promega) in a 20-μl reaction mixture containing 10 U of avian myeloblastosis virus RT (Promega). The PCR was carried out with 2.5 U of *Taq* polymerase (Perkin-Elmer) and primers 2 and 3 (each at a concentration of 0.1 μM) in a 100-μl reaction mixture. The seminested PCR was performed with 2.5 U of *Taq* polymerase (Perkin-Elmer) and primers 2 and F2 (each at a concentration of 0.1 μM) in a 100-μl reaction mixture. Amplifications (PCR and seminested PCR) were performed for 30

TABLE 1. Efficiency of extraction methods as evaluated by RT-PCR detection of enteric viruses in mussel extracts

| Extraction method | No. of positive samples/total no. of samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Astrovirus | | HAV | | Poliovirus | | Total | |
| | Undiluted samples | 10-Fold-diluted samples | Undiluted samples | 10-Fold-diluted samples | Undiluted samples | 10-Fold-diluted samples | Undiluted samples | 10-Fold-diluted samples |
| Borate | 3/3 | 2/3 | 3/3 | 0/3 | 2/3 | 2/3 | 8/9 | 4/9 |
| Glycine | 3/3 | 0/3 | 1/3 | 0/3 | 2/3 | 2/3 | 6/9 | 2/9 |
| Saline beef | 3/3 | 1/3 | 1/3 | 0/3 | 1/3 | 1/3 | 5/9 | 2/9 |
| Saline beef-Freon | 2/2 | 1/2 | 2/3 | 0/3 | 3/3 | 1/3 | 7/8 | 2/8 |

TABLE 2. Efficiency of extraction methods as evaluated by RT-PCR detection of enteric viruses in concentrates prepared from mussel extracts

| Extraction method | No. of positive samples/total no. of samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Astrovirus | | HAV | | Poliovirus | | Total | |
| | Undiluted samples | 10-Fold-diluted samples | Undiluted samples | 10-Fold-diluted samples | Undiluted samples | 10-Fold-diluted samples | Undiluted samples | 10-Fold-diluted samples |
| Borate | 9/12 | 4/12 | 10/12 | 8/12 | 12/12 | 3/12 | 31/36 | 15/36 |
| Glycine | 7/12 | 4/12 | 12/12 | 9/12 | 11/11 | 3/12 | 30/35 | 16/36 |
| Saline beef | 0/18 | 0/18 | 3/18 | 2/18 | 13/18 | 6/18 | 16/54 | 8/54 |
| Saline beef-Freon | 11/18 | 0/15 | 10/18 | 0/18 | 11/18 | 4/18 | 32/54 | 4/51 |

cycles consisting of denaturation for 30 s at 94°C, primer annealing for 45 s at 50°C, and elongation for 1 min at 72°C.

For HAV, 2-μl RNA extracts were reverse transcribed for 1 h at 37°C by using 100 U of Moloney murine leukemia virus RT (Gibco BRL) and 1 μM primer E. The PCR was then carried out in a 25-μl mixture containing 2.5 μl of cDNA, 0.5 μM primer D, 0.5 μM primer E, each deoxynucleoside triphosphate at a concentration of 200 μM, and 0.625 U of *Taq* DNA polymerase (Gibco BRL). Denaturation was performed for 7 min at 94°C, and this was followed by 35 cycles of amplification consisting of denaturation for 30 s at 94°C, annealing for 90 s at 62°C, and extension for 90 s at 62°C. A final extension step was performed for 5 min at 72°C.

Poliovirus and astrovirus amplified products were analyzed by electrophoresis on 2% agarose gels, and HAV amplified products were analyzed by electrophoresis on a 9% polyacrylamide gel. Amplified products were visualized by UV illumination after the gels were stained with ethidium bromide. Differences in percentages of positive results were analyzed by the chi-square or Fisher's exact test.

Three distinct mussel contamination experiments were performed with the same viral suspensions for each virus. The shellfish were contaminated by natural means (uptake from water) because direct injection of virus into shellfish homogenates has little resemblance to natural conditions, under which virus extraction is probably more difficult.

A total of 214 samples were analyzed; the samples included 71 samples analyzed for astrovirus RNA (11 extraction experiments and 60 extraction-concentration experiments), 72 samples analyzed for HAV RNA (12 extraction experiments and 60 extraction-concentration experiments), and 71 samples analyzed for poliovirus RNA (12 extraction experiments and 59 extraction-concentration experiments). Each RT-PCR experiment was performed more than once. When only extracts were compared (Table 1), no significant differences were observed among the four extraction methods either with undiluted samples ($P = 0.3$) or with 10-fold-diluted samples ($P = 0.61$). Table 2 shows the results of the extraction experiments as evaluated by RT-PCR detection of enteric viruses in mussel concentrates. Table 3 combines the data in Tables 1 and 2 and compares the efficiencies of the four extraction methods. The extraction methods could be arranged in the following decreasing order of efficiency: borate buffer, glycine solution, saline beef-Freon, and saline beef. A comparison by the chi-square test of the overall results in Table 3 showed that both the borate buffer and glycine solution extraction methods were significantly more effective than the other two methods since glycine was significantly more effective than saline beef ($P < 0.0001$) and saline beef-Freon ($P = 0.01$). In a previous study, in which a cell culture was used to detect viruses, extraction was more efficient with beef extract than with 0.05 M glycine (8). In our study, the saline beef extraction method resulted in detection of only 14% (3 of 21) of the astrovirus RNA-positive samples and 19% (4 of 21) of the HAV RNA-positive samples, which highlights the fact that beef extract interferes with molecular detection methods (25).

A comparison of the concentration results, including the results obtained for all crude and detoxified undiluted concentrates, showed that astrovirus, HAV, and poliovirus RNAs were detected in 9 of 24 (37.5%), 15 of 24 (62.5%), and 17 of 23 (74%), respectively, OF concentrates, in 12 of 24 (50%), 16 of 24 (67%), and 19 of 24 (79%), respectively, PEG 6000 concentrates, and in 6 of 12 (50%), 4 of 12 (33%), and 11 of 12 (92%), respectively, PEG 8000 concentrates. For the three viruses, the percentages of positive samples were not significantly different when PEG 6000 and OF undiluted concentrates were compared (for astrovirus, $P = 0.38$; for HAV, $P = 0.76$; for poliovirus, $P = 0.67$) or when PEG 6000 and OF 10-fold-diluted concentrates were compared (for astrovirus, $P = 0.56$; for HAV, $P = 0.77$; for poliovirus, $P = 0.16$). PEG 8000 concentrate results were not included in this comparison because PEG 8000 concentration was not performed after borate buffer and glycine solution extractions, which were the most efficient extraction methods.

The efficiencies of the 20 combinations of extraction and concentration methods used for astrovirus, HAV, and polio-

TABLE 3. Efficiency of extraction methods as evaluated by RT-PCR detection of enteric viruses in mussel extracts and in concentrates prepared from mussel extracts

| Extraction method | No. of positive samples/total no. of samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Astrovirus | | HAV | | Poliovirus | | Total | |
| | Undiluted samples | 10-Fold-diluted samples | Undiluted samples | 10-Fold-diluted samples | Undiluted samples | 10-Fold-diluted samples | Undiluted samples | 10-Fold-diluted samples |
| Borate | 12/15 | 6/15 | 13/15 | 8/15 | 14/15 | 5/15 | 39/45 | 19/45 |
| Glycine | 10/15 | 4/15 | 13/15 | 9/15 | 13/14 | 5/15 | 36/44 | 18/45 |
| Saline beef | 3/21 | 1/21 | 4/21 | 2/21 | 14/21 | 7/21 | 21/63 | 10/63 |
| Saline beef-Freon | 13/20 | 1/17 | 12/21 | 0/21 | 14/21 | 5/21 | 39/62 | 6/59 |

TABLE 4. Efficiency of viral extraction-concentration combinations as evaluated by RT-PCR detection of astrovirus, HAV, and poliovirus in mussel samples

| Extraction method | Concentration method | No. of positive samples/total no. of samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Astrovirus | | HAV | | Poliovirus | | Total | |
| | | Undiluted samples | 10-Fold-diluted samples | Undiluted samples | 10-Fold-diluted samples | Undiluted samples | 10-Fold-diluted samples | Undiluted samples | 10-Fold-diluted samples |
| Borate | OF (LH20)[a] | 2/3 | 0/3 | 3/3 | 2/3 | 3/3 | 0/3 | 8/9 | 2/9 |
| Borate | OF | 3/3 | 3/3 | 3/3 | 3/3 | 3/3 | 1/3 | 9/9 | 7/9 |
| Borate | PEG 6000 (LH20)[a] | 1/3 | 0/3 | 1/3 | 0/3 | 3/3 | 1/3 | 5/9 | 1/9 |
| Borate | PEG 6000 | 3/3 | 1/3 | 3/3 | 3/3 | 3/3 | 1/3 | 9/9 | 5/9 |
| Glycine | OF (LH20)[a] | 1/3 | 0/3 | 3/3 | 2/3 | 2/2 | 0/3 | 6/9 | 2/9 |
| Glycine | OF | 1/3 | 0/3 | 3/3 | 3/3 | 3/3 | 0/3 | 7/9 | 3/9 |
| Glycine | PEG 6000 (LH20)[a] | 2/3 | 0/3 | 3/3 | 2/3 | 3/3 | 1/3 | 8/9 | 3/9 |
| Glycine | PEG 6000 | 3/3 | 1/3 | 3/3 | 2/3 | 3/3 | 2/3 | 9/9 | 5/9 |
| Saline beef | OF (LH20)[a] | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 1/3 | 3/9 | 1/9 |
| Saline beef | OF | 0/3 | 0/3 | 0/3 | 0/3 | 1/3 | 0/3 | 1/9 | 0/9 |
| Saline beef | PEG 6000 (LH20)[a] | 0/3 | 0/3 | 3/3 | 2/3 | 1/3 | 0/3 | 4/9 | 2/9 |
| Saline beef | PEG 6000 | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 2/3 | 3/9 | 2/9 |
| Saline beef | PEG 8000 (LH20)[a] | 0/3 | 0/3 | 0/3 | 0/3 | 2/3 | 1/3 | 2/9 | 1/9 |
| Saline beef | PEG 8000 | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 2/3 | 3/9 | 2/9 |
| Saline beef-Freon | OF (LH20)[a] | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/9 | 0/9 |
| Saline beef-Freon | OF | 2/3 | 0/3 | 3/3 | 0/3 | 2/3 | 1/3 | 7/9 | 1/9 |
| Saline beef-Freon | PEG 6000 (LH20)[a] | 0/3 | 0/3 | 1/3 | 0/3 | 1/3 | 0/3 | 2/9 | 0/9 |
| Saline beef-Freon | PEG 6000 | 3/3 | ND[b] | 2/3 | 0/3 | 2/3 | 0/3 | 7/9 | 0/6 |
| Saline beef-Freon | PEG 8000 (LH20)[a] | 3/3 | 0/3 | 1/3 | 0/3 | 3/3 | 0/3 | 7/9 | 0/9 |
| Saline beef-Freon | PEG 8000 | 3/3 | 0/3 | 3/3 | 0/3 | 3/3 | 3/3 | 9/9 | 3/9 |

[a] A Sephadex LH20 gel filtration detoxification step was included.
[b] ND, not done.

virus RT-PCR detection are shown in Table 4. All of the concentrates obtained with the borate buffer-OF, borate buffer-PEG 6000, glycine solution-PEG 6000, and saline beef-Freon–PEG 8000 combinations gave positive RT-PCR results consistently for the three viruses when undiluted preparations were analyzed. When 10-fold-diluted concentrates were analyzed, the borate buffer-OF method gave the highest detection rate. The saline beef extraction method yielded very low RT-PCR detection rates whatever concentration procedure was used. For the latter method, a further purifying step in which Freon was used to remove inhibitors improved PCR detection of both astrovirus ($P = 0.0009$) and HAV ($P = 0.01$).

PEG 6000 precipitation and OF are the methods used most widely to concentrate enteric viruses from shellfish (20). Lees et al. reported good poliovirus recovery and substantial reductions in RT-PCR inhibitors when PEG 6000 was used (16). In our study, addition of a concentration step following viral extraction further improved, albeit not significantly, the viral RNA detection rates for the borate buffer and glycine solution extraction methods but not for the saline beef and saline beef-Freon extraction methods. No significant difference in viral RNA detection was observed when the PEG 6000 precipitation and OF concentration methods were compared. Recently, Jaykus et al. proposed a method based on double precipitation with PEG and Pro-cipitate that results in small-volume concentrates suitable for sensitive detection by viral infectivity and RT-PCR amplification methods (14). However, this technique is time-consuming, and our goal was to compare techniques used for RT-PCR detection only.

Shellfish virological analysis is often hampered by the toxicity of shellfish concentrates for cell cultures. It has been proposed that a detoxification technique based on Sephadex LH20 gel filtration can reduce this cytotoxicity (5). Our results showed that this procedure is not suitable before RT-PCR detection of the three viruses used in this study. Other procedures, such as processing hepatopancreatic tissue rather than whole shellfish tissue, may overcome the inhibitory effects on RT-PCR (24). Using RT-PCR, we detected astrovirus, HAV, and poliovirus RNAs in 18 of 30 (60%), 20 of 30 (67%), and 26 of 30 (87%), respectively, undiluted crude concentrates, compared with 9 of 30 (30%), 15 of 30 (50%), and 21 of 29 (72%), respectively, undiluted Sephadex LH20-detoxified concentrates. The percentage of positive samples was significantly higher for crude concentrates than for detoxified concentrates ($P = 0.004$), probably because of viral losses subsequent to viral adsorption onto the Sephadex gel during the detoxification step.

In conclusion, the borate buffer-PEG 6000, borate buffer-OF, and glycine solution-PEG 6000 precipitation combinations were the most efficient combinations for detecting astrovirus, HAV, and poliovirus in both undiluted samples and 10-fold-diluted samples. Conversely, most of the combinations that included saline beef extraction were unable to detect any HAV or astrovirus RNA-positive samples. The three efficient procedures described here will soon be evaluated in our laboratories for detection of astrovirus, HAV, poliovirus, and other important enteric viruses, such as small round structured viruses, in naturally polluted shellfish, which may have high inhibitory potential and low levels of viral contamination.

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